SYMPOSIUM ON BACTERIAL ENDOTOXINS[1]

IV. Immunological Aspects of the Host Reaction to Endotoxins

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The nature of the biological activity of the bacterial endotoxins continues to puzzle investigators in this field. After nearly a half-century of research, it is still not clear whether these agents kill and produce their other effects by virtue of some intrinsic pharmacological activity, or whether the reactions to endotoxins are mediated by immunological or hypersensitivity phenomena. The latter hypothesis has much to recommend it. Skin reactions to intradermal test doses of endotoxin in man and rabbits resemble tuberculin reactions in their evolution and appearance. The Shwartzman reaction occurring in such areas can be mimicked with tuberculin in hypersensitive animals, as can the pyrogenic effect of endotoxins. Shock and death following large parenteral doses of endotoxin are reminiscent of "tuberculin shock" in hypersensitive animals, and in general it can be said that most of the biological effects of endotoxins in normal animals can be reproduced with foreign protein antigens in specifically hypersensitive animals. It is tempting to consider the possibility, then, that endotoxins are "toxins" only in the sense that tuberculoprotein or other antigens are toxic for the hypersensitive animal (7). A necessary corollary, of course, is that so-called normal animals possess a natural allergy or hypersensitivity to these ubiquitous gram-negative bacterial antigens and, more specifically, an allergy of the "delayed" or tuberculin type.

Unfortunately, there are several considerations which argue strongly against this hypothesis. In the first place, it seems clear that the antigenicity of bacterial endotoxins resides in the polysaccharide portion of the molecule, but it has never been demonstrated that polysaccharide antigens as a class can induce or elicit delayed hypersensitivity. In the second place, polysaccharide fractions resulting from gentle hydrolysis of the endotoxin molecule are usually found to be devoid of biological activity although they retain their antigenicity. Furthermore, if reactivity to endotoxins depended on prior sensitization, one would expect embryos and newborn or germ-free adult animals to be unreactive; in fact, chick embryos (6), newborn rabbits (3) and germ-free rats (B. Zweifach, *unpublished data*) are all susceptible to the lethal effect of endotoxins. Finally, R. A. Good (*unpublished data*) has recently found that patients with Hodgkin's disease, who characteristically fail to exhibit delayed hypersensitivity reactions, nevertheless exhibit quite normal skin reactivity to bacterial endotoxins.

Although there are, then, striking similarities between the reactions of normal animals to endotoxins and the reactions of hypersensitive animals to foreign protein antigens, reactivity to endotoxins cannot be interpreted in terms of conventional bacterial allergy acquired by the individual. It is possible that some form of altered reactivity to these antigens does exist on a genetic basis, and is expressed even in the embryo by some mechanism analogous to isohemagglutinin production. Species differences in reactivity to endotoxins might reflect either differences in survival advantage, differences in ecological relationships, or both.

Another, and perhaps more likely, possibility is that the similarities between hypersensitivity reactions and reactions to endotoxins merely represent final common pathways of tissue reaction, triggered in different ways but expressed in a common phenomenology. In any event, it will be interesting to determine whether susceptibility to endotoxins is in fact genetically determined and to study the reactions of the embryo and newborn before contact with bacterial antigens has occurred.

Turning from delayed hypersensitivity to circulating antibodies, we again find a number of observations suggesting an immunological basis for endotoxin action. The effect in vitro of endotoxin on blood clotting, described by McKay, Shapiro, and Shanberge (4), has attracted interest

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because of the prominence of thrombotic phenomena in some of the effects in vivo of endotoxin, notably the local and generalized Shwartzman phenomenon. This effect has been shown to be antibody mediated (5) and can be mimicked by other antigen-antibody complexes. So, too, in the case of the substantial stimulation of respiration of leukocytes by addition of endotoxin in vitro (8).

Although it has been more difficult to demonstrate the mediation by antibody of effects in vivo of endotoxin, the accelerated cutaneous reactivity which follows a single injection of endotoxin correlates well with the prompt antibody response (3). This antibody response has some of the characteristics of an anamnestic response, and cross-reacting antibodies also appear. That is, stimulation by a single dose of one bacterial endotoxin leads to the prompt appearance of accelerated skin reactivity to that endotoxin and to others as well, the serum antibody titers against the homologous and heterologous endotoxins showing a parallel increase. The basis for the cross reactivity appears to be similar in dideoxyhexose residues on the polysaccharides of the endotoxins.

Another phenomenon in vivo in which these cross-reacting antibodies may be involved is the "tolerance" seen after repeated injections of endotoxin. Freedman (2) has been able to transfer this tolerance passively with plasma or serum; as in the tolerant donors, the transferred tolerance extended to other endotoxins as well (1), and it would be worthwhile to determine whether this cross tolerance could be accounted for on the basis of serological cross reactivity between the endotoxins used.

It appears, therefore, that whether or not the primary biological activity of endotoxins hinges on their antigenicity, circulating antibodies play a role in modification of the host response to these agents. Under some circumstances, increased antibody levels appear to confer an "immediate" or Arthus-like skin reactivity in the rabbit; under others, increased antibody levels may aid in the rapid clearance of circulating endotoxin by the reticulo-endothelial system, resulting in a diminished biological activity or "tolerance." Studies of the complicated situation in the adult laboratory animal, whose reactivity to endotoxins is almost certainly modified by his previous contact with endotoxins, could certainly be profitably complemented by studies on reactivity of the fetus and newborn, and we must know more of the qualitative as well as quantitative aspects of the immunological response to endotoxins before we shall know how to evaluate properly their role in determining the effects in vivo of endotoxins.

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